DNA 7500 MAINTENANCE

A. SCOPE

A.1 This procedure outlines the monthly maintenance of the 7500 real-time PCR instrument.

B. QUALITY CONTROL

B.1 Protective gloves, scrubs, and a lab coat must be worn at all times when performing this procedure to prevent contamination.

B.2 Bleach and ethanol can cause fluorescence causing more unwanted background in the test. Therefore, only clean dirty wells with bleach and ethanol as a last resort. Always clean with distilled water first and then retest.

C. SAFETY

C.1 Protective gloves, scrubs, and a lab coat must be worn at all times when performing this procedure. Additionally, if ethanol or bleach is being used, eye protection (e.g. safety glasses or a face shield) must be worn.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 70% Ethanol (Decontamination)

D.2 Bleach (Decontamination)

E. EQUIPMENT

E.1 AB 7500

F. PROCEDURES

F.1 Performing the Background Calibration on the 7500's

F.1.1 Open a new plate document. Select:

F.1.1.1 Assay → Background

F.1.1.2 Container → 96-Well Clear

F.1.1.3 Template → Blank Document

F.1.1.4 Enter your name in the operator field

F.1.1.5 Click "Finish"

F.1.2 In the SDS software, save the file as:

F.1.2.1 Background-DDMMYY-initials (or something similar).

F.1.2.2 Save the 7500-A file in the file directory C:\7500 A Monthly Maintenance.

F.1.2.3 Save the 7500-B file in the file directory C:\7500 B Monthly Maintenance.

F.1.2.4 Save the 7500-C file in the file directory C:\7500 C Monthly Maintenance.

F.1.3 Load an empty 96 well plate that has been sealed with optical adhesive film into the 7500.

F.1.4 Select the "instrument" tab.

F.1.4.1 Click "Start".

F.1.5 When the run is completed, you will see a popup window displaying: "The run completed successfully.

F.1.5.1 Click "OK".

F.1.6 Click the analysis button (green triangle) to extract the background.

F.1.7 In the plate document, select the "Results" tab, and then select the "Spectra" tab.

F.1.8 Select all wells of the plate document.

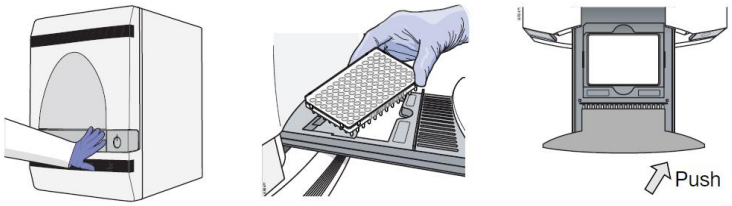
F.1.9 Inspect the raw data for irregular spectral peaks that exceed 72,000 FSU.

F.1.9.1 If one or more wells produce raw spectra that exceed the specified FSU, the background plate or sample block could contain a fluorescent contaminant.

F.1.9.2 Determine the source of the contamination by inspecting one row of wells at a time, followed by each well in the row that contains the irregular peak until the well that is potentially contaminated is evident.

F.2 Cleaning the Sample Wells

F.2.1 Remove the plate and tray holder from the instrument.



F.2.2 Close the tray.

F.2.3 Manually raise the block :

F.2.3.1 **Instrument→ Instrument Maintenance Manager.**

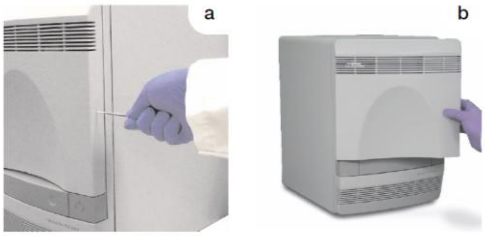
F.2.3.2 In the ROI tab of the Instrument maintenance Manager, click **Start Manual Calibration.**

F.2.3.3 In the ROI Inspector dialog box, click **Move Block.**

F.2.3.4 When the ROI inspector dialog box displays "Block Down", click **Done**

F.2.4 Power off the 7500.

F.2.5 Open the access door to the 7500 system.



F.2.6 Lift the Latch, and then push the heated cover door to the back of the instrument.

F.2.7 Clean the contaminated well(s) using a small volume of deionized water:

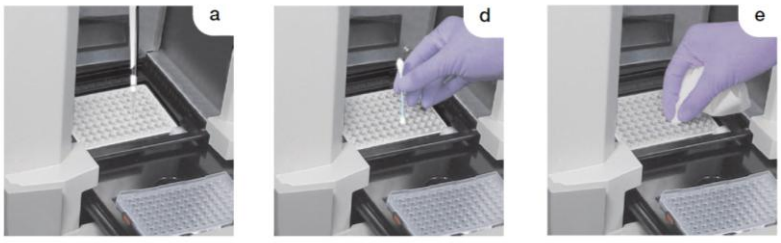
F.2.7.1 Pipette a small volume of deionized water into each contaminated well.

F.2.7.2 Pipette the water up and down several times to rinse the well.

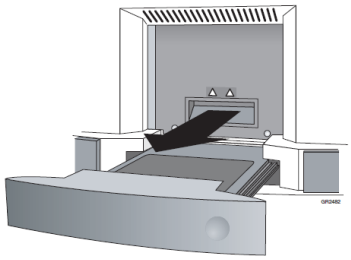
F.2.7.3 Pipette the water to a waste basket.

F.2.7.4 Using a cotton swab, clean the inside of each contaminated well.

F.2.7.5 Using a lint free cloth, absorb the excess deionized water.



F.2.8 Pull the heated cover door to the front of the instrument. Lift the latch, then secure the heated cover door to the cross bar.



F.2.9 Power on the 7500 system.

F.2.10 Run a new background plate.

F.2.11 If the contamination remains, repeat the procedure using an ethanol solution to clean, followed with a deionizied water wash.

F.2.12 If the contamination still remains, repeat the procedure using a 10% bleach solution followed by a deionized water wash.

F.2.13 If the contamination still remains, the instrument will be removed from service and Life Technologies support will be contacted.

F.3 Performing the Function Tests

F.3.1 Create a new document.

F.3.2 In the new document, click "Finish" to accept the default parameters.

F.3.3 In the menu bar, select "Instrument" → "Function Test".

F.3.4 In the Function Test dialog box, check to see that the function test passed.

F.3.5 Click "All Tests".

F.3.6 Examine the Pass/Fail column, and click "OK".

F.3.7 If everything passes, then select "File" → "Close".

F.3.8 When the software prompts you to save the plate document, click "No".

G. INTERPRETATION GUIDELINES

G.1 If any of the function tests fail, power off the instrument, wait 30 seconds, then power on the instrument and repeat the function test. If the function test fails a second time, the instrument will be removed from service and Life Technologies will be contacted.

H. REFERENCES

H.1 7500 Manual